

## ABSTRACT

SPECTRAL CALIBRATION OF FLUORESCENT  
POLYNUCLEOTIDE SEPARATION APPARATUS

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10 The invention relates to methods, compositions, and systems for calibrating a  
fluorescent polynucleotide separation apparatus. One aspect of the invention is multiple color  
calibration standards and their use. A multiple color calibration standard is a mixture of at  
least two polynucleotides of different length, wherein each of the polynucleotides is labeled  
with a spectrally distinct fluorescent dye. Another aspect of the invention is to produce total  
emission temporal profiles of multiple color calibration standards for use in calibrating  
fluorescent polynucleotide separation apparatus. The peaks corresponding to the  
fluorescently labeled polynucleotides in the total emission temporal profile may be detected  
using a peak detector that is driven by changes in the slopes of the total emission temporal  
profile. Calibration of fluorescent polynucleotide separation apparatus, with various  
embodiments of the methods of the invention, includes the step of identification of the  
labeled polynucleotides of the multiple color calibration standards. The process of spectral  
calibration of a fluorescent polynucleotide separation apparatus using a multiple color  
calibration standard may include the step of the estimating (extracting) of the dyes' reference  
spectra, using information from the peak detection process performed on the total emission  
temporal profile. Other aspects of the invention include systems for separating and detecting  
fluorescently labeled polynucleotides, wherein the system is designed for spectral calibration  
in accordance with the subject calibration methods employing multiple color calibration  
standards. Another aspect of the invention is methods and compositions for detecting the  
flow of electrical current through a separation channel of a fluorescent polynucleotide  
separation apparatus. These methods and compositions employ monitoring dyes. Monitoring  
dyes are fluorescent dyes that are spectrally distinct from the dye on the polynucleotide  
intended to convey genetic information, e.g., fluorescent polynucleotide sequencing reaction  
products.